MICROBIC VIRULENCE AND HOST SUSCEPTIBILITY IN PARATYPHOID-ENTERITIDIS INFECTION OF WHITE MICE.

V. THE EFFECT OF DIET ON HOST RESISTANCE.

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One of us (Webster) has shown that the general non-specific resistance of mice bred at the Rockefeller Institute includes important elements associated with heredity and that, by selective breeding, the general resistance of a mouse population may be altered. We wish now to describe some experiments which demonstrate the effect of diet on host susceptibility to infection and intoxication.

For some years the mice in the breeding room of the Rockefeller Institute have received a diet consisting of a daily ration of baker's bread soaked in fresh pasteurized Grade B milk warmed to at least 60-70°C., supplemented by two weekly feedings of an oatmeal and buckwheat mixture and one weekly feeding of dog biscuit. On this diet the animals thrive and breed well and are to all appearances in excellent health.

It was thought desirable to compare the resistance of these mice with that of similar mice raised on some other stock diet in general use. Consequently, a so called normal diet for mice and rats, advocated by E. V. McCollum, was chosen which consists of the following ingredients: wheat 67.5 per cent, casein 15 per cent, milk powder 10 per cent, NaCl 1 per cent, CaCO₃ 1.5 per cent, and butter fat 5 per cent. This diet was prepared by mixing the finely ground whole wheat with the inorganic salts, casein, commercial milk powder, and the clear fat from melted sweet butter. Small quantities were continually avail-

2 This particular formula for the McCollum complete diet was obtained through a personal communication from Dr. E. V. McCollum.
able to the mice in porcelain salve jars. A constant supply of fresh water was also provided.

Experiment 1 was planned to compare the resistance of mice fed on the bread and milk diet with that of similar mice fed on the McCollum diet.

*Experiment 1.*—Twelve young female mice, bred at the Rockefeller Institute and in early pregnancy, were chosen and caged in separate boxes in the breeding room. Six of these mice were maintained on the regular bread and milk diet and six were changed to the McCollum diet prepared from vitamine-free casein and purified butter fat. The litters of the first group were therefore nourished by McCollum diet mothers while those of the second group were fed by bread and milk diet mothers. After weaning time, the respective diets were continued, the

![Text-fig. 1](image)

first group, ten mice, receiving McCollum diet and the second group, ten mice, the Institute bread and milk diet throughout the entire experiment.

When the young mice had reached a weight of 16 to 18 gm. each, they were placed in separate jars and inoculated by stomach tube with 4,500,000 mouse typhoid bacilli, Type II, grown in plain broth pH 7.4 for 16 hours. Autopsies were performed on those dying and cultures taken from heart’s blood and intestinal contents. Mortality curves for the two groups were plotted, together with the standard control curve constructed by Webster for Rockefeller Institute mice fed on the regular breeding room diet (Text-fig. 1.).

*Experiment 2.*—The twelve females described in Experiment 1 were kept on their respective diets and mated a second time. The $F_1$ generation was fed and

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1 These materials were obtained through the kindness of Dr. P. A. Levene and his department.


treated as in Experiment 1. One group, twenty-six mice, continued to receive McCollum diet after weaning and the second group, twenty-six mice, was fed the bread and milk diet.

When the young had reached a weight of 16 to 18 gm. each, they were injected as before with a dose of 3,600,000 bacilli and caged separately for observation. The respective diets were continued; fatal cases were autopsied and cultured for the mouse typhoid bacilli. Mortality curves of the two groups of mice, together with the standard control curve for Institute mice, are plotted in Text-fig. 2.

The results of these two experiments show that mice fed on the McCollum diet are more resistant to mouse typhoid infection than are similar mice nourished on a bread and milk diet.

**Experiment 3.**—The experiment was repeated a third time under somewhat different conditions. The pregnant females from the Rockefeller Institute stock, instead of being kept in the breeding room under the care of the regular attendant, were cared for by us in another room. A great effort was made to have this care in every way comparable to the care given the mice in the breeding room. The pregnant females were again divided into two diet groups, one receiving the regular breeding room diet, the other a McCollum complete diet, the latter prepared from commercial materials instead of the carefully purified materials used in Experiments 1 and 2. The small mice were weaned when 3 weeks old, and were all continued on the same diet that their mothers had received during pregnancy, and lactation. When they were 8 to 10 weeks old, thirty-three from the McCollum diet series and thirty-six from the control diet series, all weighing at least 18 gm., were assembled in separate battery jars and were each given by stomach tube 0.005 cc. of a 24 hour broth culture of M. T. II (7,000,000 organisms) in a 0.5 cc. volume. They were observed through the following 8 weeks, the deaths charted and cultures taken at autopsy from the spleen and feces, to determine the presence of M. T. II. The results of the experiment are presented graphically in Text-fig. 3.
Text-fig. 3 shows the standard control curve for the mice from the breeding room, the curve for the control diet series described above, and the curve for the corresponding McCollum diet series. It will be seen that the curve for the control diet series runs somewhat lower than the standard control curve. Nevertheless, it is obvious that in this experiment, as well as in Experiments 1 and 2, the mice fed on the McCollum complete diet were far more resistant to the infection than were those fed on our regular breeding room diet, despite the fact that before the experiment was begun the control diet mice were to all appearances as healthy as were those on the McCollum diet.

One of us has shown that if a group of mice from the Rockefeller Institute breeding room is given per os a sufficient dose of bichloride of mercury a mortality curve can be obtained which resembles the curves obtained with fixed doses of M. T. II. It has further been shown that the resistance to mercury bichloride of a mouse population that has survived infection with M. T. II is greater than that of normal mice, indicating that the resistance of these individuals is to some extent non-specific. Since we had observed that the resistance of our mice to mouse typhoid infection can be modified through diet, we wished to know whether under similar circumstances they would also prove to be more resistant to bichloride of mercury given by mouth. Accordingly, a small number of the mice described above were used in an experiment with bichloride of mercury. For such a test it is essential that mice of very nearly equal weight be used.

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6 Webster, L. T., J. Exp. Med., 1923, xxxviii, 45.
Experiment 4.—Ten mice were selected from the control diet series and nine from the McCollum diet series, all weighing 18 to 19 gm. Each of these mice received, by stomach tube, 0.5 cc. of a 1:200 dilution of bichloride of mercury in distilled water (0.0025 gm.), individuals of the two series receiving it alternately. The mice were observed over a period of 48 hours, deaths being recorded hourly. The results are shown graphically in Text-fig. 4.

It will be seen from Text-fig. 4 that the mice on the McCollum diet were distinctly more resistant to the injection of bichloride of mercury than those on the control diet. The McCollum diet curve is very much more gradual and the final mortality rate of these mice is 15 per cent below that of the mice on the control diet. It is to be regretted that at the time this experiment was performed larger numbers of mice on the two diets were not available. Nevertheless, when taken in conjunction with the three experiments on the effect of diet upon mouse typhoid infection, the results obtained in Experiment 4 would seem to be significant.

Additional evidence in support of the non-specific nature of the resistance conferred by the McCollum diet was obtained from an experiment kindly made for us by Dr. J. Bronfenbrenner in which he compared the resistance of McCollum diet-fed mice and bread and milk diet-fed mice to botulinus toxin.

Experiment 5.—Although the mice used in this experiment were the survivors from Experiment 3 and might therefore be considered as a selected group, both the McCollum diet series and the bread and milk diet series had undergone the same treatment and were therefore suitable for further investigation. Fifteen McCollum diet mice and seven bread and milk diet mice received 1 M.L.D. of botulinus
toxin intraperitoneally and twelve McCollum diet mice and eight bread and milk diet mice received 2 M.L.D. intraperitoneally. The percentage mortality in the two groups of mice is plotted in Text-figs. 5 and 6.

The curves (Text-figs. 5 and 6) indicate that the mice fed on the McCollum diet were distinctly more resistant to *botulinus* toxin than were similar mice maintained on a bread and milk diet. Although the final mortality is the same for each group, the lag, as in the experiment with mercury bichloride, is far greater among the mice fed on the McCollum diet than among those fed on a bread and milk diet.

This experiment, taken in conjunction with Experiment 4 and the previous work reported, constitutes further evidence that the so called general resistance of the host may be largely non-specific in nature.
DISCUSSION.

The literature relating to the effects of diets on health and disease contains few specific facts of an experimental nature. However, various investigators in the relatively new field of quantitative dietetics have expressed the opinion that the response of animals to inoculation with bacteria and certain poisons is affected by dietary deficiencies in salt, vitamin, protein, and other food substances.

We have examined two sets of mice of the same race and stock from the Rockefeller Institute breeding room as regards their response to a strain of mouse typhoid bacilli, mercury bichloride, and botulinus toxin. One set was from the general stock which had been fed on the regular breeding room diet, as given above, for many generations without any evidence of apparent deficiency. The second set of mice, also from the regular Institute stock, differed only in being fed on McCollum's diet. These mice throve well, of course, but not perceptibly better than the first set. And yet the far greater resistance of the McCollum diet-fed animals both to Bacillus pestis cavia, botulinus toxin, and mercury bichloride is striking.

To explain this disparity on the basis of dietary effects in the nature of deficiencies is to make assumptions on factors that are very subtle. It is true that such subtle deficiencies are postulated by McCollum and Cramer. Possibly the future may reveal the existence of a deficiency operating insidiously and over a long period of time through many generations of animals. Until such time as an undoubted experimental basis is secured for this conception, we prefer merely to record our experimental results without discussing their ultimate meaning and explanation. The fact is, however, indisputable that profoundly striking effects on the behavior of mice to an infectious bacterium, a bacterial toxin, and a chemical poison can be produced by feeding, in the absence of any manifestations that are attributable to dietary deficiencies as now recognized.

CONCLUSIONS.

White mice from the Rockefeller Institute breeding room fed on a McCollum complete diet, consisting of whole wheat (67.5 per cent), casein (15 per cent), milk powder (10 per cent), NaCl (1 per cent), CaCO₃ (1.5 per cent), and butter fat (5 per cent) are more resistant to mouse typhoid infection, mercury bichloride intoxication, and botulinus toxin than are similar mice fed on bread and pasteurized milk supplemented by an oatmeal and buckwheat mixture and dog biscuit.